pBI

287 Bfr BI
287 Nsi I
287 Ppu 10I

Sca I 6720

Bpm I 6327

1285 Xcm I
1288 Bse RI

Afw NI 5760

pBI

+

7159 base pairs
Unique Sites

1748 Bsp EI
1841 Pml I
1956 Apa BI
1956 Bsl API
2124 Bsu 36I
2174 Bst XI

Sap I 6226

Pst I 4776
Sal I 4770
BamHI 4764
Xma I 4759
Sma I 4759
Sty I 4749
Nco I 4749
Bst DSI 4749
Sfi I 4747
Msc I 4746
Hpa I 4570

2880 Dra III
2985 Nae I
2986 Ngo MIV

3289 Nru I
3293 Ecl 136II
3293 Sac I
3384 Mlu I
3465 Ppu MI
3515 Rle AI

Bst BI 4026
Bsa BI 3797

Oligo 160

| gagagtagtaacaaaggtc | AAAGACAGTTGACTGTATCGCCG GAA TTT AT

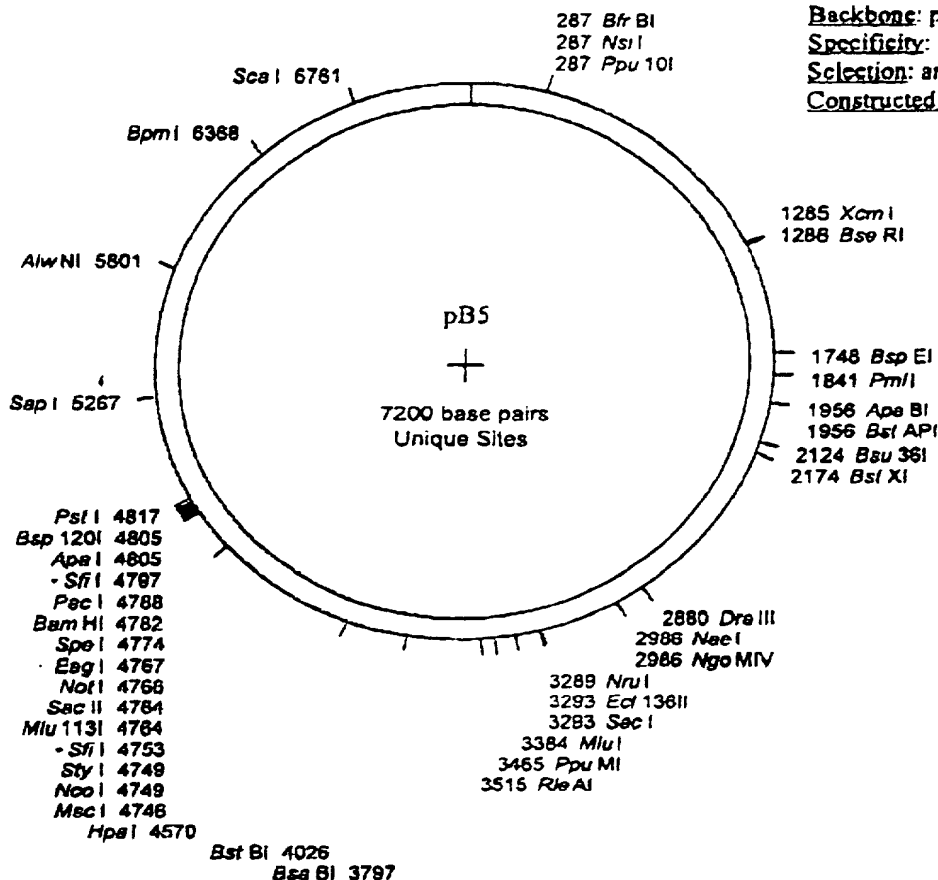|	Sfi I		Sma I		BamH I		Sal I		Pst I	
G	GCC ATG GAG	GCC	CCG	GGG	ATC	CGT	CGA	CCT	GCA	GCC
	Nco I									

Oligo 161

AAG CTA ATT | ccgggcgaattcttatg

Oligo 160 5' GAGAGTAGTAACAAAGGTC 3'
Oligo 161 5' CATAAGAAATTCGCCCGG 3'

FIGURE 1

pB5²

pB5

7200 base pairs
Unique Sites

287 Bfr BI
287 Nsi I
287 Ppu 10I

Sca I 6761

Bpm I 6368

AlwN I 5801

Sap I 5267

1285 Xcm I
1288 Bse RI

1748 Bsp EI
1841 Pml I

1956 Ape BI
1956 Bsf APf

2124 Bsu 36I
2174 Bst XI

2880 Dra III
2986 Nae I
2986 Ngo MIV

3289 Nru I
3293 Ecl 136II
3293 Sec I
3384 Mlu I
3465 Ppu MI
3515 Rle AI

Pst I 4817
Bsp 120I 4805
Apa I 4805
• Sfi I 4797
Pac I 4788
Bam HI 4782
Spe I 4774
• Eag I 4767
Not I 4766
Sac II 4764
Mlu 113I 4764
• Sfi I 4753
Sty I 4749
Nco I 4749
Msc I 4746
Hpa I 4570

Bst BI 4026
Bsa BI 3797

Oligo 160

| gagagtagtaacaaaggtc | AAAGACAGTTGACTGTATCGCCG GAA TTT ATG

                          Sfi I   Sac II   Spe I   Bam HI
GCC ATG GCC GCA GGG GCC GCG GCC GCA CTA GTG GGG ATC C
         Nco I   Not I

          STOP   Sfi I   Pst I
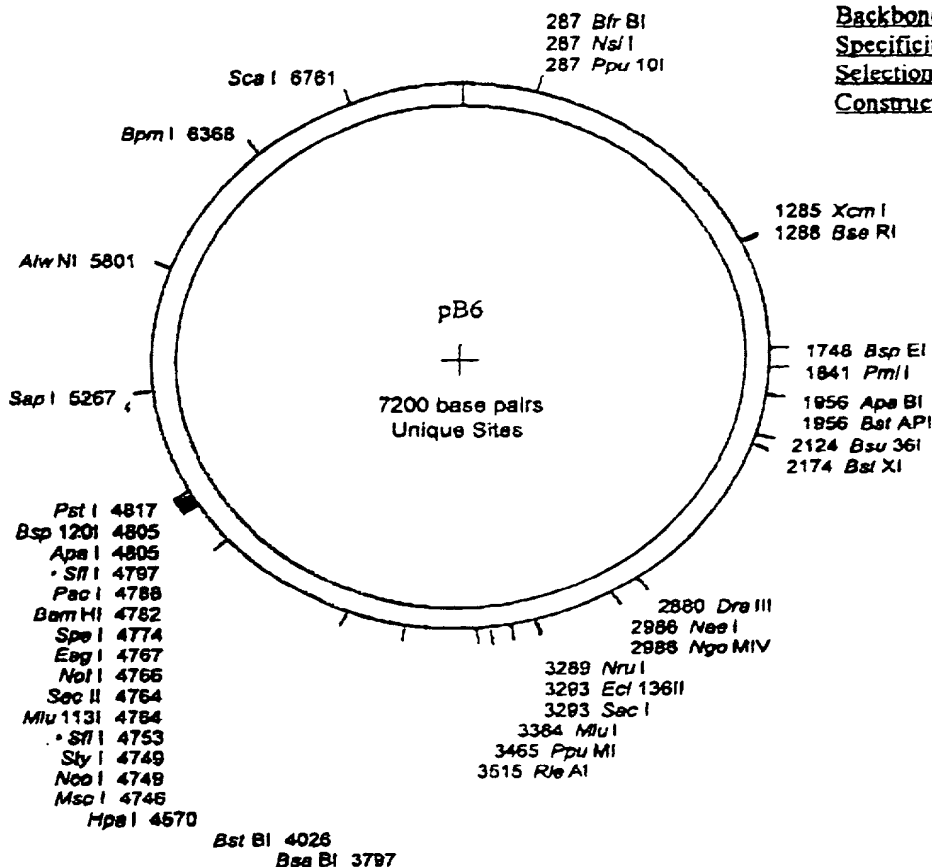TT AAT |TAA| GGG CCA CTG GGG CCC CTC GAC CTG CAG CCA
          Pac I

                         Oligo 161
AGC TAA TT | ccgggcgaatttcttatg |

Oligo 160 5' GAGAGTAGTAACAAAGGTC 3'
Oligo 161 5' CATAAGAAATTCGCCCGG 3'
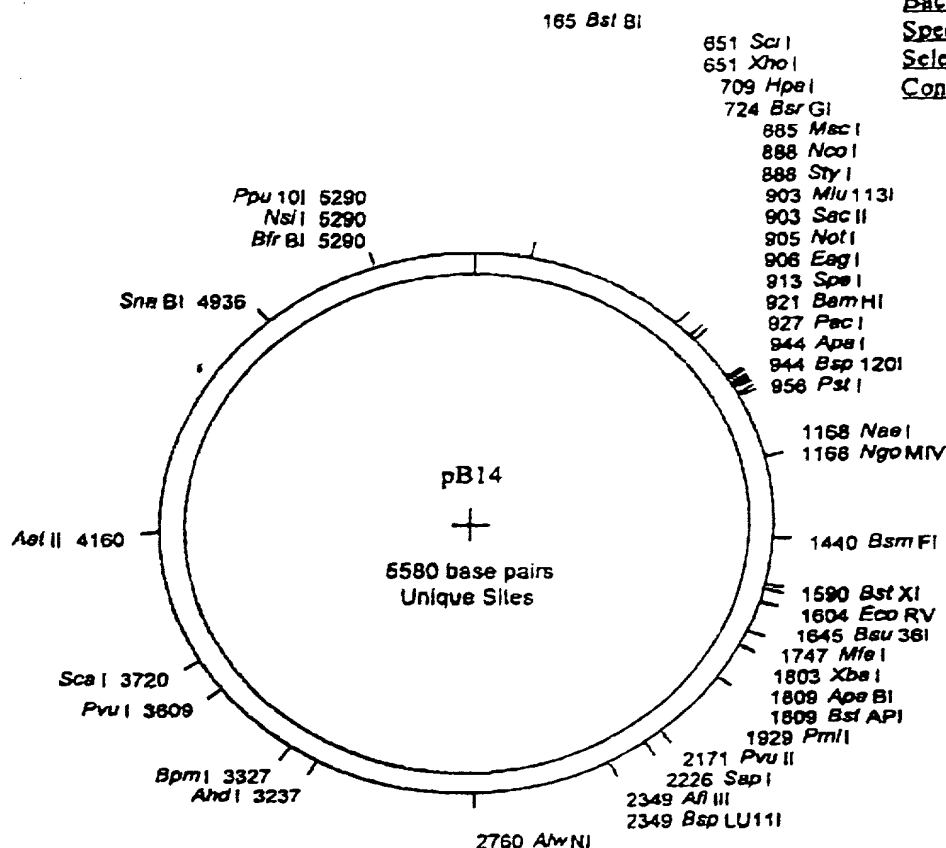
FIGURE 2

pB6

287 Bfr BI
287 Nsi I
287 Ppu 10I

Sca I 6761

Bpm I 6368

Afw NI 5801

Sap I 5267

pB6

+

7200 base pairs
Unique Sites

1285 Xcm I
1288 Bse RI

1748 Bsp EI
1841 Pml I
1956 Aps BI
1956 Bst API
2124 Bsu 36I
2174 Bst XI

Pst I 4817
Bsp 120I 4805
Apa I 4805
• Sfi I 4797
Pac I 4788
Bam HI 4782
Spe I 4774
Eag I 4767
Not I 4766
Sac II 4764
Mlu 113I 4764
• Sfi I 4753
Sty I 4749
Nco I 4749
Msc I 4746
Hpa I 4570

Bst BI 4026
Bsa BI 3797

2880 Dra III
2986 Nae I
2988 Ngo MIV
3289 Nru I
3293 Ecl 136II
3293 Sac I
3384 Mlu I
3465 Ppu MI
3515 Rle AI

Oligo 160

| gagagtagtaacaaaggtc | AAAGACAGTTGACTGTATCGCCG GAA TTT ATG

                    Sfi I   Sac II   Spe I   Bam HI

GCC ATG GCC GGA CGG GCC GCG GCC GCA CTA GTG GGG ATC C
    Nco I   Not I

       STOP   Sfi I   Apa I   Pst I

TT AAT | TAA | GGG CCA CTG GGG CCC CTC GAC CTG CAG CCA
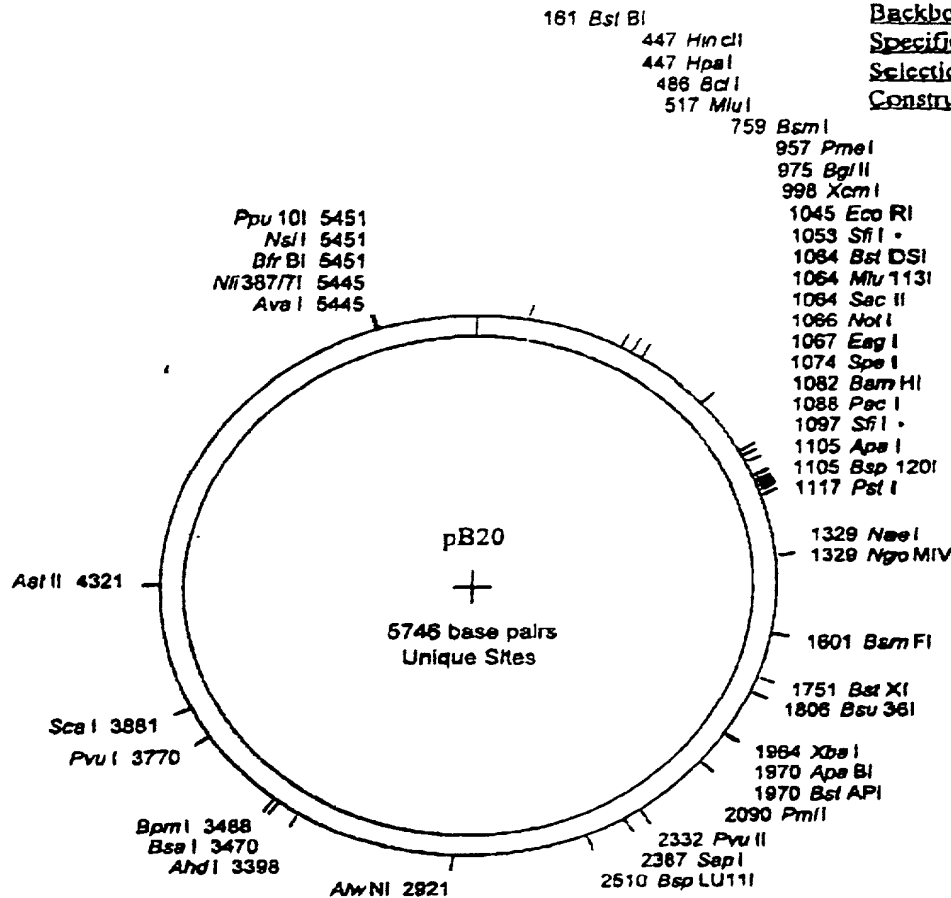 Pac I

Oligo 161

AGC TAA TT | ccgggcgaatttcttatg |

Oligo 160 5' GAGAGTAGTAACAAAGGTC 3'
Oligo 161 5' CATAAGAAATTCGCCCGG 3'

FIGURE 3

pB13

165 *Bst* BI

651 *Sca* I
651 *Xho* I
709 *Hpa* I
724 *Bsr* GI
885 *Msc* I
888 *Nco* I
888 *Sty* I
903 *Mlu* 113I
903 *Sac* II
905 *Not* I
906 *Eag* I
913 *Spe* I
921 *Bam* HI
927 *Pac* I
944 *Apa* I
944 *Bsp* 120I
956 *Pst* I

Ppu 10I 5290
Nsi I 5290
Bfr BI 5290

Sna BI 4936

1168 *Nae* I
1168 *Ngo* MIV

Aat II 4160

1440 *Bsm* FI

pB13

5580 base pairs
Unique Sites

1590 *Bst* XI
1604 *Eco* RV
1645 *Bsu* 36I
1747 *Mfe* I
1803 *Xba* I
1809 *Apa* BI
1809 *Bst* API
1929 *Pml* I

Sca I 3720
Pvu I 3609

2171 *Pvu* II
2226 *Sap* I
2349 *Afl* III
2349 *Bsp* LU11I

Bpm I 3327
Ahd I 3237

2760 *Alw* NI

Oligo 160

| gagagtagtaacaaaggtc | AAAGACAGTTGACTGTATCGCCG GAA TTT ATG

```
                      Sfi I                Sac II               Spe I           Bam HI
GCC  ATG  GCC  GCA  GGG  GCC  GCG  GCC  GCA  CTA  GTG  GGG  ATC  C
      Nco I                          Not I
```

```
      STOP                 Sfi I                              Pst I
TT  AAT  TAA  GGG  CCA  CTG  GGG  CCC  CTC  GAC  CTG  CAG  CCA
      Pac I
```

Oligo 161

AGC TAA TT | ccgggcgaatttcttatg |

Oligo 160 5' GAGAGTAGTAACAAAGGTC 3'
Oligo 161 5' CATAAGAAATTCGCCCGG 3'

FIGURE 4

pB14

165 Bsi BI

651 Sca I
651 Xho I
709 Hpa I
724 Bsr GI
885 Msc I
888 Nco I
888 Sty I
903 Mlu 113I
903 Sac II
905 Not I
906 Eag I
913 Spe I
921 Bam HI
927 Pac I
944 Apa I
944 Bsp 120I
956 Pst I

Ppu 10I 5290
Nsi I 5290
Bfr BI 5290

Sna BI 4936

1168 Nae I
1168 Ngo MIV

1440 Bsm FI

Aat II 4160

pB14
+
5580 base pairs
Unique Sites

1590 Bst XI
1604 Eco RV
1645 Bsu 36I
1747 Mfe I
1803 Xba I
1809 Ape BI
1809 Bsf API
1929 Pml I

Sca I 3720
Pvu I 3609

2171 Pvu II
2226 Sap I
2349 Afl III
2349 Bsp LU11I

Bpm I 3327
Ahd I 3237

2760 Alw NI

Oligo 160

| gagagtagtaacaaaggtc | AAAGACAGTTGACTGTATCGCCG GAA TTT ATG

           Sfi I   **Sac II**   **Spe I**   **Bam HI**

GCC ATG GCC GGA CGG GCC GCG GCC GCA CTA GTG GGG ATC C

  Nco I   **Not I**

    STOP   **Sfi I**   **Apa I**   **Pst I**

TT AAT |TAA| GGG CCA CTG GGG CCC CTC GAC CTG CAG CCA

  Pac I

Oligo 161

AGC TAA TT |ccgggcgaatttcttatg|

Oligo 160 5' GAGAGTAGTAACAAAGGTC 3'
Oligo 161 5' CATAAGAAATTCGCCCGG 3'

FIGURE 5

pB20 [6]

Alias: pLex10NS2
Application: 2HY (bait)
Backbone: pLex10 (pB9)
Specificity: Sfi-oriented
Selection: ampicillin
Constructed by: LD

161 Bst BI
447 Hin cII
447 Hpa I
486 Bcl I
517 Mlu I
759 Bsm I
957 Pme I
975 Bgl II
998 Xcm I
1045 Eco RI
1053 Sfi I ·
1064 Bst DSI
1064 Mlu 113I
1064 Sac II
1066 Not I
1067 Eag I
1074 Spe I
1082 Bam HI
1088 Pac I
1097 Sfi I ·
1105 Apa I
1105 Bsp 120I
1117 Pst I

1329 Nae I
1329 Ngo MIV

1601 Bsm FI

1751 Bst XI
1806 Bsu 36I

1964 Xba I
1970 Apa BI
1970 Bst API
2090 Pml I

2332 Pvu II
2387 Sap I
2510 Bsp LU11I

AlwNI 2921

Bpm I 3488
Bsa I 3470
Ahd I 3398

Sca I 3881
Pvu I 3770

Aat II 4321

Ppu 10I 5451
Nsi I 5451
Bfr BI 5451
Nli 387/7I 5445
Ava I 5445

pB20

5746 base pairs
Unique Sites

EcoR I		Sfi I					Not I		Spe I		BamH I	
GAA	TTC	GGG	GCC	GGA	CGG	GCC	GCG	GCC	GCA	CTA	GTG	GGG ATC C

Sac II

STOP

				Sfi I					Pst I	
TT AAT	TAA	GGG	CCA	CTG	GGG	CCC	CTC	GAC	CTG	CAG

Pac I

FIGURE 6

pP1

Bcl I 7932
Spe I 7817
Xba I 7533
Sna BI 7194
Hpa I 6552
Nru I 6146
Blp I 6111
Bae I 6056
Sse 8647I 5778
Bst EII 5622
Cla I 5506
Age I 5407
Eco RV 4909
Bsr GI 4536
Dra III 4092

pP1
8134 base pairs
Unique Sites

377 Bsa HI
435 Sca I
846 Bsa I
913 Ahd I
1392 AlwNI
1928 Sap I
2059 Tth 111I
2157 Bsm BI
2575 Bsg I
2764 Rsr II
2905 Sex AI
2934 Mlu I
3094 Sfi I
3096 Nco I
3106 Sma I
3106 Xma I
3111 BamH I
3117 Eco RI
3123 Ban II
3123 Ecl 136II
3123 Sac I
3143 Scl I
3143 Xho I

ABS1

`cgtttggaatcactacagg` GATGTTTAATACCACTACAATGGATGATGTATATAACTATCTATT

JC90   **Bgl II**

`cgatgatgaagataccccaccaaa` CCCAAAAAAAGAGATCTGTATGGCTTACCCATACGATGTTCCAG

                   Sfi I   **Sma I**   **BamH I**

ATTACGCTAGCTTGGGTGGTCATATGGCC ATG GAG GCC CCG GGG ATC CGA ATT

                           Nco I

Sac I   **Xho I**   **Bgl II**

CGA GCT CGA CTA GCT AGC TGA CTC GAG AGA TCT ATGAAT

`cgtagatactgaaaaaccoc` GCAAGTT `cacttcaactgtgcatcgtg` `caccatctcaatttc`

      162   **ABS2**   **53**

ABS1 5' CGTTTGGAATCACTACAGG 3'
JC90 5' CGATGATGAAGATACCCCACCAAA 3'
162   5' GGGGTTTTTCAGTATCTACG 3'
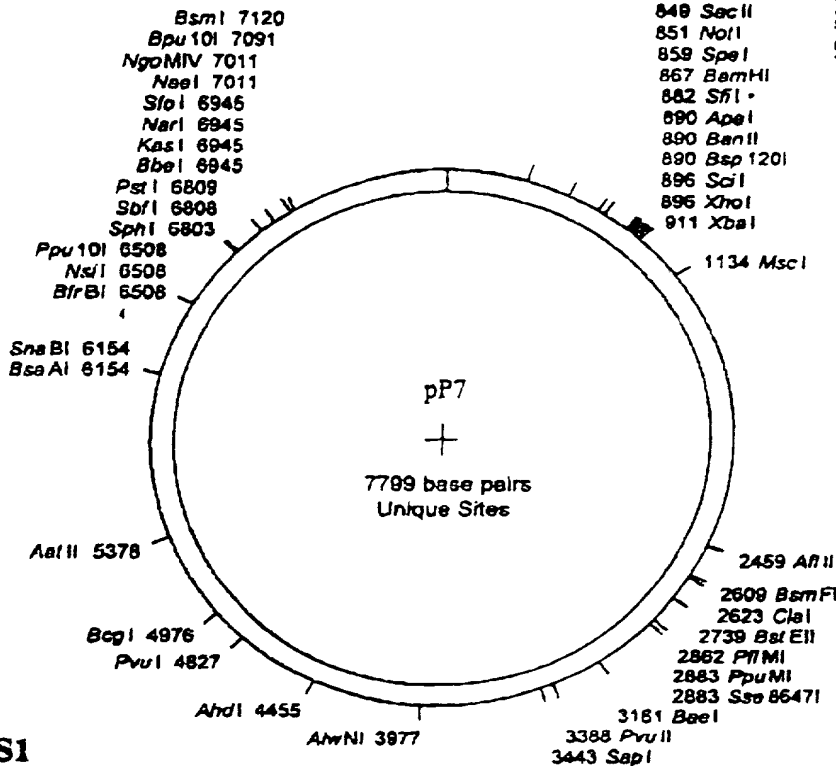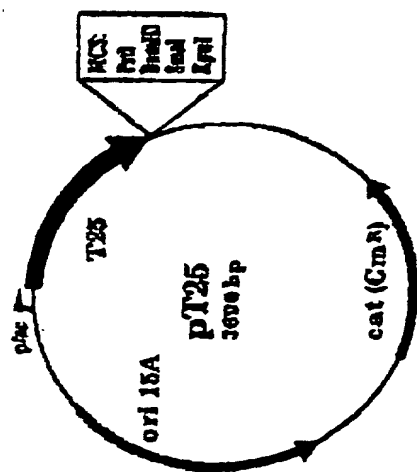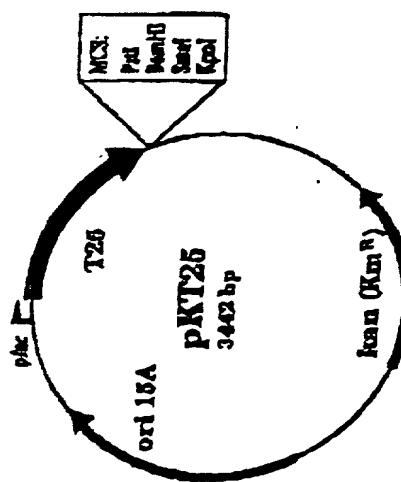ABS2 5' CACGATGCACAGTTGAAGTG 3'
53   5' GAAATTGAGATGGTGCACGATGCAC 3'
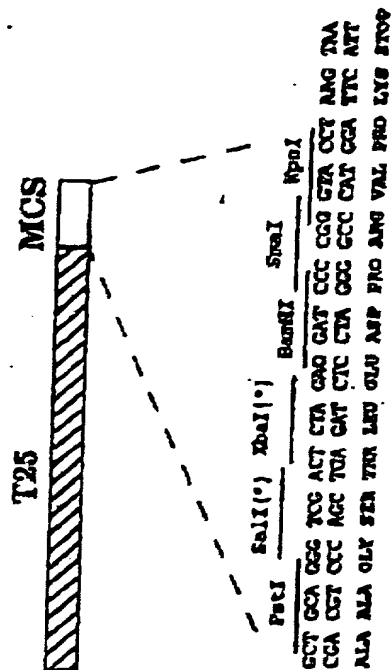
FIGURE 7

pP2[8]

Bcl I 7947
Xba I 7548
Sna BI 7209
377 Bsa HI
435 Sca I
846 Bsa I
913 Ahd I

Hpa I 6567

pP2

1392 Ahw NI

Nru I 6161
Blp I 6126
Bae I 6071

8149 base pairs
Unique Sites

1928 Sap I
2059 Tth 111 I
2157 Bsm BI

Sse 8647 I 5793
Bst EII 5637
Cla I 5521
Age I 5422

2575 Bsg I
2764 Rsr II
2905 Sex AI
2934 Mlu I
3096 Nco I
3100 Sfi I •
3111 Mlu 113 I
3111 Sac II
3129 Bam HI
3135 Pac I
3144 Sfi I •
3152 Apa I
3152 Ban II
3152 Bsp 120 I
3158 Scl I
3158 Xho I

Eco RV 4924
Bsr GI 4551
Dra III 4107

ABS1

CG `cgtttggaatcactacagg` GATGTTTAATACCACTACAATGGATGATGTATATAACTATCTATT

JC90

`cgatgatgaagatacccaccaaa` CCCAAAAAAAGAGATCTGTATGGCTTACCCATACGATGTTCCAG

                             Bgl II

                                              Sfi I   **Sac II**

ATTACGCTAGCTTGGGTGGTCATATGGCC ATG GCC GCA GGG GCC GCG GCC GCA
                                           Nco I

            BamH I   **Pac I**

CTA GTG GGG ATC CTT AAT `TAA` GGG CCA CTG GGG CCC CTC GAG AGA TCT
                            Stop

ATGAAT `cgtagatactgaaaaacccc` GCAAGTT `cacttcaactgtgcatcgtg` `caccatctcaatttc`
                   162   **ABS2**   **53**

ABS1 5' CGTTTGGAATCACTACAGG 3'

JC90 5' CGATGATGAAGATACCCCACCAAA 3'

162 5' GGGGTTTTTCAGTATCTACG 3'

ABS2 5' CACGATGCACAGTTGAAGTG 3'

53 5' GAAATTGAGATGGTGCACGATGCAC 3'

FIGURE 8

pP3

Bcl I 7947
Xba I 7648
Sna BI 7209
Hpa I 6567
Nru I 6161
Blp I 6126
Bse I 6071
Sse 8647I 5793
Bst EII 5637
Cla I 5521
Age I 5422
Eco RV 4924
Bsr GI 4551
Dra III 4107

377 Bsa HI
435 Sca I
846 Bse I
913 Ahd I
1382 Alw NI
1928 Sap I
2059 Tth 111I
2157 Bsm BI
2575 Bsg I
2764 Rsr II
2905 Sex AI
2934 Mlu I
3096 Nco I
3100 Sfi I ·
3111 Mlu 113I
3111 Sac II
3129 Bam HI
3135 Pac I
3144 Sfi I ·
3152 Ape I
3152 Ban II
3152 Bsp 120I
3156 Sd I
3158 Xho I

pP3

8149 base pairs
Unique Sites

ABS1

CG |cgtttggaatcactacagg| GATGTTTAATACCACTACAATGGATGATGTATATAACTATCTATT

JC90   **Bgl II**
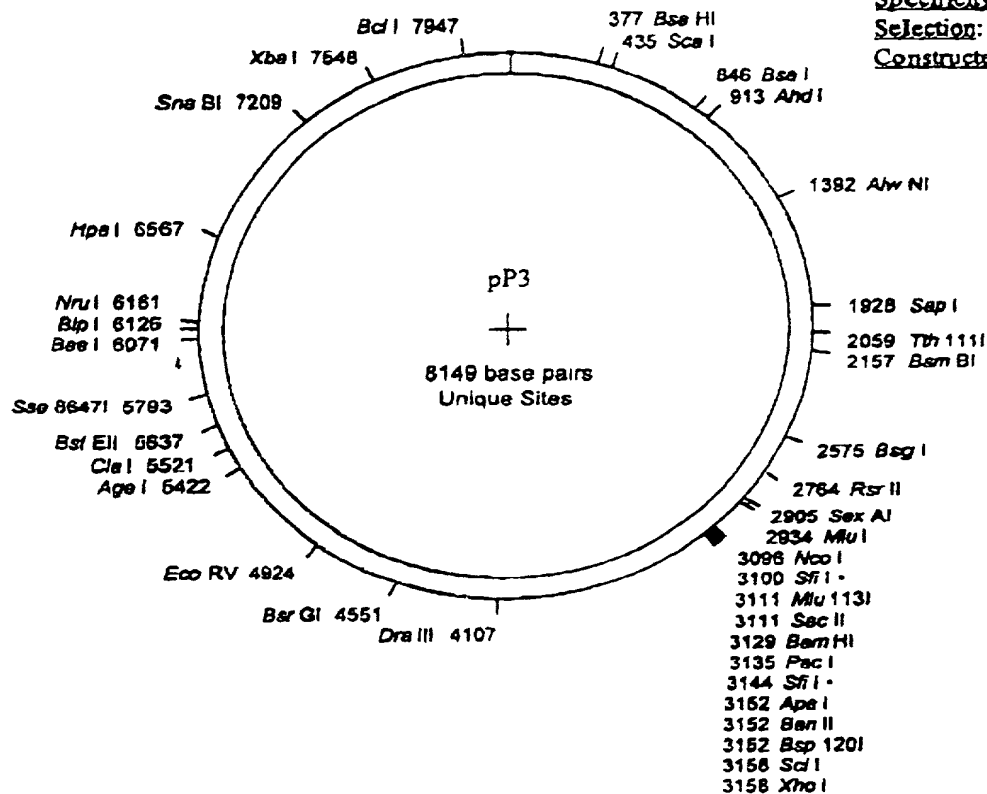
|cgatgatgaagataccccaccaaa| CCCAAAAAAAGAGATCTGTATGGCTTACCCATACGATGTTCCAG

                                              Sfi I   **Sac II**

ATTACGCTAGCTTGGGTGGTCATATGGCC ATG GCC GGA CGG GCC GCG GCC GCA
                                    Nco I

   BamH I   **Pac I**
CTA GTG GGG ATC CTT AAT |TAA| GGG CCA CTG GGG CCC CTC GAG AGA TCT
                         Stop

ATGAAT |cgtagatactgaaaaacccc| GCAAGTT |cacttcaact|gtgcatcgtg| caccatctcaatttc|
          162   **ABS2**   53

ABS1 5' CGTTTGGAATCACTACAGG 3'
JC90 5' CGATGATGAAGATACCCCACCAAA 3'
162   5' GGGGTTTTTCAGTATCTACG 3'
ABS2 5' CACGATGCACAGTTGAAGTG 3'
53   5' GAAATTGAGATGGTGCACGATGCAC 3'

FIGURE 9

pP6 10

358 Bsg I
547 Rsr II
688 Sex AI
717 Mlu I
834 Nco I
838 Sfi I ·
849 Mlu113I
849 Sac II
851 Not I
859 Spe I
867 BamHI
882 Sfi I ·
890 Apa I
890 Ban II
890 Bsp 120I
896 Sci I
896 Xho I
911 Xba I

Alias: pGAD3S2XNS1
Application: 2HY (prey)
Backbone: pGAD3S2X
Specificity: Sfi non-oriented
Selection: ampicillin
Constructed by: SW

Bsm I 7120
Bpu10I 7091
NgoMIV 7011
Nae I 7011
Sfo I 6946
Nar I 6946
Kas I 6945
Bbe I 6945
Pst I 6809
Sbf I 6809
Sph I 6803
Ppu10I 6508
Nsi I 6508
BfrBI 6508

SnaBI 6154
Bsa AI 6154

pP6

+

7799 base pairs
Unique Sites

1134 Msc I

Aat II 5376

2459 Afl II
2609 BsmF I
2623 Cla I
2739 Bst EII
2882 Pfl MI
2883 Ppu MI
2883 Sse 8647I
3161 Bae I
3388 Pvu II
3443 Sap I

Bag I 4876
Pvu I 4827

Ahd I 4455

AlwNI 3977

ABS1

| cgtttggaatcactacagg | GATGTTTAATACCACTACAATGGATGATGTATATAACTATCTATT

JC90

| cgatgatgaagatacccaccaaa | CCCAAAAAAAGAGATCCTAGAACTA

           Sfi I   **Sac II**   **Spe I**   **Bam HI**

GCC ATG GCC GCA GGG GCC GCG GCC GCA CTA GTG GGG ATC C

     Nco I   **Not I**

    STOP   **Sfi I**   **Xbo I**   **Xba I**

TT AAT |TAA| GGG CCA CTG GGG CCC CTC GAG |TAG| CTA GTG TCT AGA

                                 STOP   **STOP**   **STOP**

GGCCCGGTACCCAATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGCCG TCGTTTTA

CAACGTCGTGACTGGGAAAACCCTGATCTATGAAT | cgtagatactgaaaaacccc | GCAA

                                                       162

GTT | cacttcaact gtgcatcgtg | caccatctcaatttcttc |

       ABS2   **53**

ABS1   5' CGTTTGGAATCACTACAGG 3'
JC90   5' CGATGATGAAGATACCCACCAAA 3'
162   5' GGGGTTTTTCAGTATCTACG 3'
ABS2   5' CACGATGCACAGTTGAAGTG 3'
53   5' GAAATTGAGATGGTGCACGATGCAC 3'

FIGURE 10

pP7

358 *Bsg* I
547 *Rsr* II
688 *Sex* AI
717 *Mlu* I
834 *Nco* I
838 *Sfi* I ·
849 *Mlu* 113I
849 *Sac* II
851 *Not* II
859 *Spe* I
867 *Bam* HI
882 *Sfi* I ·
890 *Ape* I
890 *Ban* II
890 *Bsp* 120I
896 *Sci* I
896 *Xho* I
911 *Xba* I

Alias: pGAD3S2XNS2
Application: 2HY (prey)
Backbone: pGAD3S2X
Specificity: Sfi oriented
Selection: ampicillin
Constructed by: SW

Bsm I 7120
Bpu 10I 7091
Ngo MIV 7011
Nae I 7011
Sfo I 6945
Nar I 6945
Kas I 6945
Bbe I 6945
Pst I 6809
Sbf I 6808
Sph I 6803
Ppu 10I 6508
Nsi I 6508
Bfr BI 6508

1134 *Msc* I

Sna BI 6154
Bsa AI 6154

pP7
+
7799 base pairs
Unique Sites

Aat II 5378

2459 *Afl* II
2609 *Bsm* FI
2623 *Cla* I
2739 *Bst* EII
2862 *Pfl* MI
2883 *Ppu* MI
2883 *Sse* 8647I
3161 *Bae* I

Bcg I 4976
Pvu I 4827

Ahd I 4455

3388 *Pvu* II
3443 *Sap* I

Ahw NI 3977

ABS1

| cgtttggaatcactacagg | GATGTTTAATACCACTACAATGGATGATGTATATAACTATCTATT |

JC90

| cgatgatgaagataccccaccaaa | CCCAAAAAAAGAGATCCTAGAACTA |

                Sfi I   **Sac II**   **Spe I**   **Bam HI**

GCC ATG GCC GGA CGG GCC GCG GCC GCA CTA GTG GGG ATC C
   Nco I   **Not I**

     STOP   **Sfi I**   **Xho I**   **Xba I**

TT AAT [TAA] GGG CCA CTG GGG CCC CTC GAG [TAG] CTA GTG TCT AGA
           STOP   **STOP** **STOP**   **STOP**

GGCCCGGTACCCAATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTA

CAACGTCGTGACTGGGAAAACCCTGATCTATGAAT | cgtagatactgaaaaacccc | GCAA
                                                           162

GTT | cacttcaac gtgcatcgtg | caccatctcaatttcttt |
     ABS2   **53**

ABS1 5' CGTTTGGAATCACTACAGG 3'
JC90 5' CGATGATGAAGATACCCCACCAAA 3'
162   5' GGGGTTTTTCAGTATCTACG 3'
ABS2 5' CACGATGCACAGTTGAAGTG 3'
53   5' GAAATTGAGATGGTGCACGATGCAC 3'

FIGURE 11

FIGURE 12

VECTORS EXPRESSING THE T25 FRAGMENT

T25   MCS

```
        PstI    SalI(*)  XbaI(*)      BamHI    XbaI      KpnI
        GCT GCA GGG TCG ACT CTA GAG GAT CCC CCG GTA CCT AAG TAA
        CGA CGT CCC AGC TGA GAT CTC CTA GGG GGC CAT GGA TTC ATT
        ALA ALA GLY SER THR LEU GLU ASP PRO ARG VAL PRO LYS STOP
```

(*) Restriction sites are not unique

MCS:
Pst
BamHI
SacI
KpnI

pT25
3696 bp

phc
T25
ori 15A
cat (CmR)

Derivative of pACYC184

T25   MCS

```
        PstI    SalI(*)  XbaI       BamHI    SmaI      KpnI
        GCT GCA GCG TCG ACT CTA GAG GAT CCC CCG GTA CCT AAG TAA
        CGA CGT CCC AGC TGA GAT CTC CTA GGG GGC CAT GGA TTC ATT
        ALA ALA GLY SER THR LEU GLU ASP PRO ARG VAL PRO LYS STOP
```
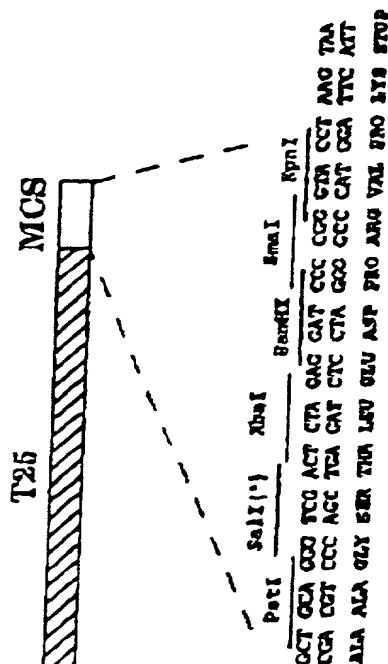
(*) Restriction site is not unique

MCS:
Pst
BamHI
SacI
KpnI

pKT25
3442 bp

phc
T25
ori 15A
kan (KmR)

Derivative of pSU40

FIGURE 12

VECTORS EXPRESSING THE T18 FRAGMENT

FIGURE 13

pCmAHL1

plac UV5

cyaA'   MCS   cat   Ori p15A

1   384

pT25

plac UV5

cyaA'   MCS   cat   Ori p15A

1   224

Pst I   BamH I   Kpn I
gctgcagggtcgactctagaggatccccgggtacctaagtaactaagaattc
A A G S T L E D P R V P K stop

pT18

plac UV5

αlacZ   cyaA'   bla   Ori C

225   399

Kpnl   MCS
tgggtaccgggccccccctcgaggtcgacggtatcgataagcttgatatcgaattcc
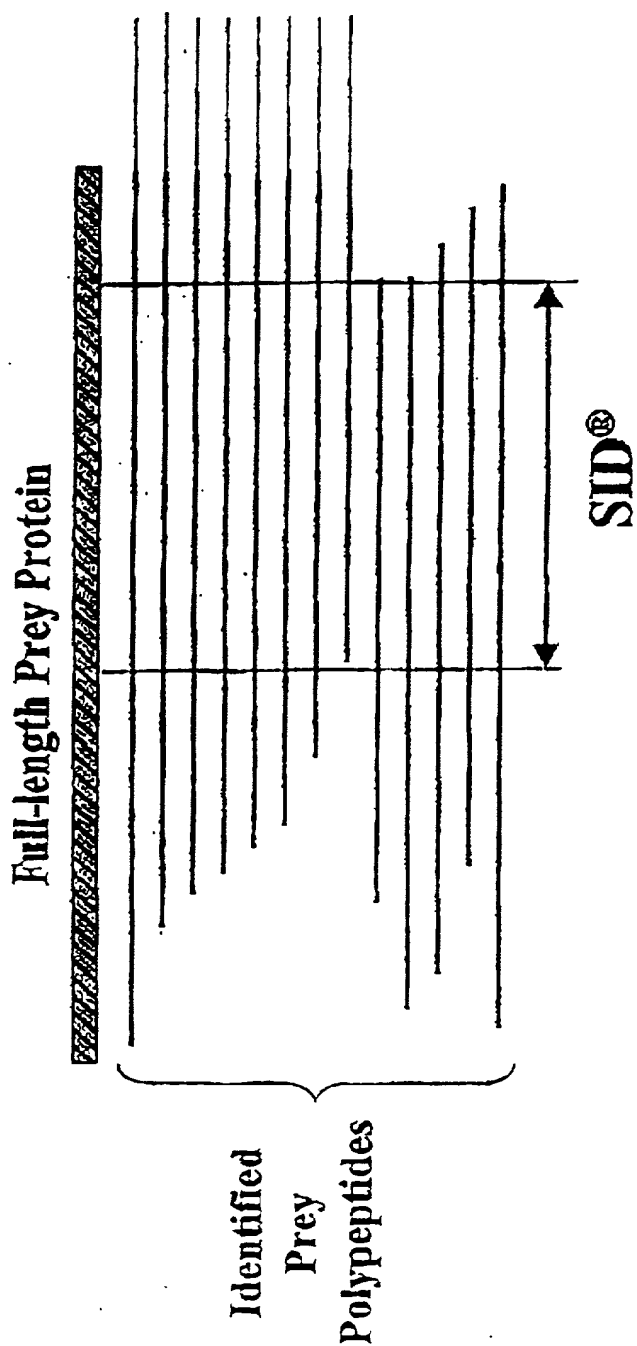W V P G P P S R S T V S I S L I S N S
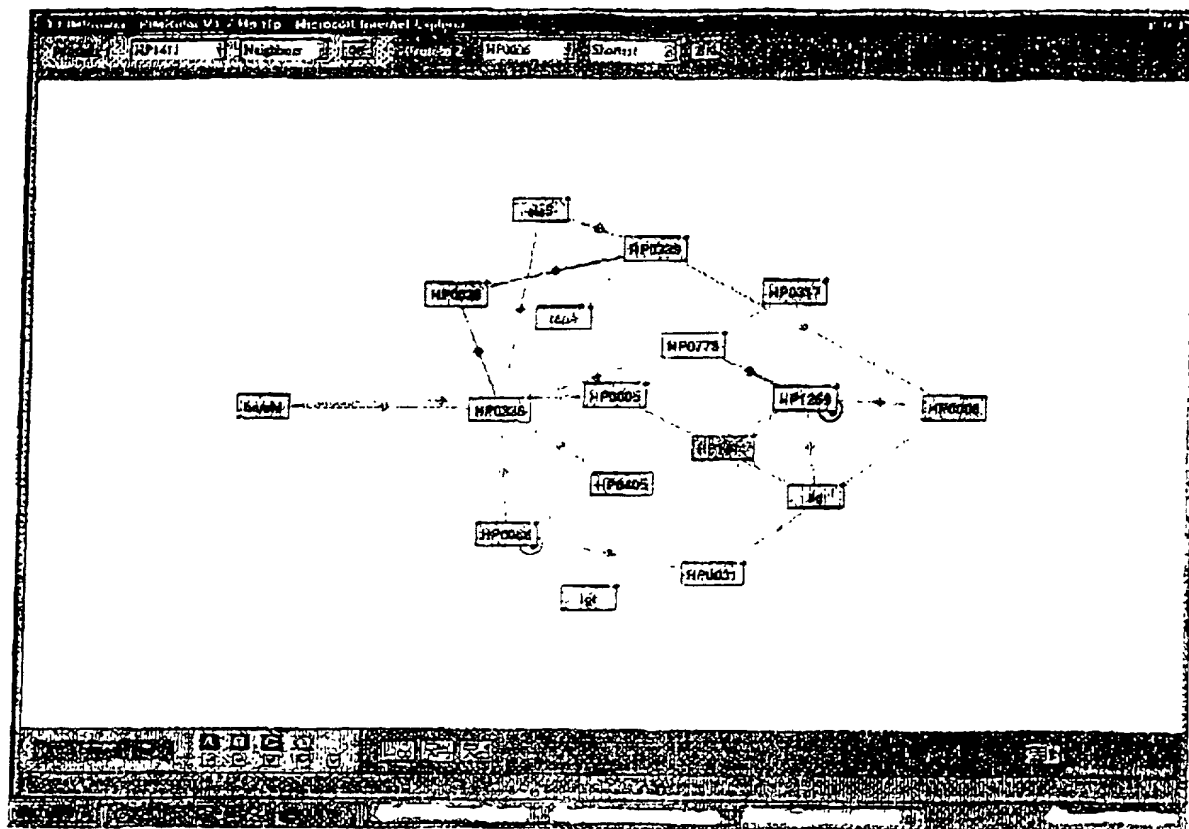
FIGURE 14

Figure 15: schematic representation of SID® determination

Figure 16 : Example of Protein Interaction Map